Disease Screening of Harbor Seals (*Phoca vitulina*) from Gertrude Island, Washington

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Introduction

As part of the Puget Sound Ambient Monitoring Program (PSAMP), the Washington Department of Fish and Wildlife (WDFW) has the responsibility for addressing the health and status of marine mammals in Puget Sound. To address this issue, harbor seals were selected as a good indicator species to monitor the overall health of marine mammals. Harbor seals were selected because they are upper-trophic-level predators that are year-round, breeding residents of Puget Sound, as well as being abundant, widely distributed, and relatively easy to study. As upper-trophic-level predators, harbor seals also provide a mechanism for monitoring how contaminants effect the health of various marine species in Puget Sound as well.

As part of PSAMP-related research efforts, WDFW has worked with researchers at the National Marine Mammal Laboratory (NMML) and other agencies to conduct: 1) aerial and ground surveys to determine status and trends of regional harbor seal populations; 2) annual capture and marking of cohorts to monitor vital parameters and reproductive success; 3) blubber biopsies to monitor temporal and spatial trends in contaminants (primarily PCBs); and 4) serologic screening of free-ranging harbor seals for various diseases. Disease screening efforts have been focused at WDFW's primary harbor seal study site at Gertrude Island (near McNeil Island). This site has the largest harbor seal haulout area in southern Puget Sound, with more than 700 seals using this site seasonally.

Determination of the health status of Puget Sound harbor seals has focused on serologic screening for a variety of diseases that have been linked with mass die-offs or illness in seals and other marine mammals from around the world. Serologic screening has been conducted for calicivirus, influenza virus, morbillivirus, leptospirosis, and brucellosis. Tissue samples were also collected and cultured for selected diseases from a number of stranded harbor seals found on Puget Sound beaches.

Methods

Capture and Handling Techniques

WDFW has conducted disease screening as part of ongoing harbor seal research efforts since 1986, with recent research activities authorized under Marine Mammal Protection Act Scientific Research Permit No. 835. The primary method of capturing harbor seals uses a beach seine technique described by Jeffries et al. (1993). Additional seals were captured by grabbing individuals following either boat or beach rushes. Once captured, seals were placed in individual hoop nets to determine weight, age, sex, and marking. Blood for serologic screening was drawn from the extradural intravertebral vein using a vacutainer adapter and an 18 gauge 1.5- to 3.5-inch needle. For blood collection, serum separator and heparinized vacutainer tubes were used. Serum was separated as soon as possible and aliquoted into 1–2 mL samples and frozen (–20 _C) pending disease screening. Heparinized samples were also frozen for bacterial blood cultures.

Collection of Specimen Materials

As part of the Northwest Marine Mammal Stranding Network, WDFW responded to reports of numerous marine mammal strandings from areas around southern Puget Sound. WDFW also has worked in cooperation with NMML to collect samples of marine mammals taken incidental to commercial fisheries. Whenever possible on live stranded marine mammals, a blood sample was drawn for health screening. From dead stranded or incidentally taken animals, blood was also collected in serum separator tubes. Blood samples were spun, serum aliquoted, and frozen. Depending on carcass condition (freshness), a selection of tissue samples was collected. For fresh or slightly decomposed animals, a thorough necropsy usually was performed. Tissue samples for histopathology were collected and stored in 10% formalin. Sections of various tissues were also collected in sterile whirl packs and frozen pending work up.

Assessment of Age Classes

Based on known pupping-season dates at Gertrude Island, and using weight/age correlations for harbor seals from British Columbia (Bigg 1969), ages of individual harbor seals were classified as nursing pup (<2 months), weaner (2–6 months), yearling (6–18 months), subadult (18–48 months), or adult (>48 months).

Diseases and Screening

Calicivirus (San Miguel Sea Lion Virus)

The first calicivirus isolated from marine mammals was San Miguel Sea Lion Virus (SMSV), recovered from two female California sea lions from the San Miguel Island rookery that had recently aborted (Smith and Boyt 1990). Since this first isolation, there have been numerous serotypes of calicivirus isolated from a variety of other marine mammals, including the northern fur seal, Steller sea lion, northern elephant seal, Pacific and Atlantic bottlenose dolphins, and walrus (Smith and Boyt 1990). Serology has shown positive titres in the bowhead whale, gray whale, fin whale, sperm whale, Sei whale, and the Hawaiian monk seal as well (Smith and Boyt 1990). The calicivirus serotype isolated from California sea lions was similar to vesicular exanthema of swine (Smith et al 1973), with clinical symptoms characterized by vesicular lesions (primarily on flippers), abortions, diarrhea, encephalitis, and death. There have been no reported cases of calicivirus in harbor seals.

The serology and virus isolation attempts were done by Oregon State University (OSU), School of Veterinary Medicine, Corvallis, OR. The serum and rectal swabs were tested for evidence of exposure to marine calicivirus serotypes as described in Smith et al. (1978) and Barlough et al. (1987).

Influenza

Influenza virus has been linked to a mass die-off of harbor seals on the New England coast in 1979 and 1980, with acute pneumonia and a secondary mycoplasma bacteria infection were the causative agents (Geraci et al. 1982). Although harbor seals seem to be most susceptible to influenza virus, other species of seals and cetaceans have been reported to be susceptible this virus as well (Webster et al. 1981; Hinshaw et al. 1986; Danner et al. 1998). The influenza virus in marine mammals has been shown to be similar to avian influenza and can be extremely virulent (Webster et al. 1981). Clinical symptoms include outwardly healthy animals that were weak and lethargic, often resulting in death. Although the influenza virus has been linked with mass die-offs of Atlantic harbor seals, it has not been reported in Pacific harbor seals.

Serological testing and attempts of isolation for influenza virus were done at St. Jude Research Hospital, Memphis, TN. Rectal and nasal swabs in culture transport media and serum were shipped overnight. The serum was tested by hemagglutinating-inhibition assay against Seal/Mass/1/80 (H7N7) and Seal/Mass/27/83 (H4N5). The rectal and nasal swabs were transferred to embryonated chicken eggs as well as MDCK cells for culture as described in Webster et al. (1981).

Morbillivirus

Morbillivirus was associated with the 1988 mass die-off of an estimated 20,000 harbor seals and several hundred grey seals in western Europe (Dietz and Harkonen 1989). The causative agent for this disease was found to be a morbillivirus similar to Canine Distemper (CDV) and subsequently named Phocine Distemper (PDV) (Mahy et al. 1988; Osterhaus and Vedder 1988; Osterhaus and Vedder 1989). Since this first reported PDV epizootic affecting seals, other strains of PDV have been identified from other pinniped and cetacean species as well (Duignan et al. 1995a; Duignan et al. 1995b). CDV has also been shown to manifest morbillivirus in marine mammals as well. Since the discovery of these potentially lethal viruses in a variety of marine mammals, screening for PDV and CDV titres has been widespread, not only in the wild but also in captive and rehabilitated animals. The clinical symptoms of CDV- and PDV-infected animals include depression, fever, cutaneous lesions, gastrointestinal dysfunction, nervous disorders, and respiratory distress (Visser et al. 1991; Visser et al. 1993). Morbillivirus has not been reported in Pacific harbor seals.

Serum samples were tested for antibodies to morbilliviruses by the National Institute of Public Health and Environmental Protection (NIPHEP), Netherlands (Dr. A.Osterhaus) in 1986 and 1989; the Department of Pathology, Microbiology and Immunology, University of California, Davis (Dr. D. King) from 1992–1994; the Department of Pathology, University of Guelph (Dr. P. Duignan) in 1993; San Jose State University, Department of Biological Sciences (Dr. J. Boothby) in 1995; and the U.S. Department of Agriculture, Plum Island (Dr. C. House) in 1996. Techniques used by the various researchers included virus-neutralization test to CDV and ELIZA; virus-neutralization tests using PDV, CDV and other morbilliviruses; and by micro titre neutralization test for antibodies to PDV.

Leptospirosis

The first report of leptospirosis in a marine mammal was from a California sea lion in 1970 (Vedros et al. 1971), and this species seems to be most affected by this disease. Epizootics that have occurred within the California sea lion population have been linked to past El Niño events. High numbers of sea lions (primarily 2–8 year old males) are found stranded on beaches with clinical symptoms which may include lethargy, depression, extreme thirst (often drinking from freshwater sources), reluctance to use rear limbs, and renal failure (Dierauf et al. 1985). Leptospirosis has been reported in northern fur seals as well (Dierauf 1990). The causative agent is *Leptospira pomona* or a similar *Leptospira* organism. Leptospirosis is zoonotic, and because of its potential health risk to humans and domestics animals (dogs, cattle, sheep, pigs, and horses), precautions should be taken when handling marine mammals that potentially carry the disease. Leptospirosis has also been linked to reproductive failure, abortions, and multiple hemorrhagic syndrome in fetuses and neonates in California sea lions and northern fur seals (Smith et al. 1974; Smith et al. 1977). Leptospirosis has not been reported from harbor seal populations.

Serological screening for leptospirosis titres was done by Oregon State University (OSU) for samples collected from 1985–1992. Since 1992, leptospirosis screening has been done by the Washington Department of Agriculture (WDA), Microbiology Laboratory in Olympia, WA. At OSU the serum was tested for titres against *Leptospira pomona*. The serum was diluted with .85% NaCl to 1:100, 1:200, 1:400, 1:1800 1:1600 and 1:3200 and tested of antibodies to *L. pomona* antigen using the microscopic agglutination test. Leptospirosis screening done at WDA included testing with *L. pomona, L. hardjo, L. grippotyphosa, L. icterohemorrhagiae* and *L. canicola*.

Brucellosis

Brucellosis is a contagious bacterial disease described in a number of mammalian species, including cattle, bison, swine, sheep, dog, and humans. It is primarily a pathogen of male and female reproductive tracts, characterized by abortion and impaired fertility (Kennedy and Miller 1993). Brucellosis can also infect and cause a variety of clinical diseases in humans (Gelfand et al. 1989).

Infection of marine mammals with brucellosis was first described in 1994 (Ross et al. 1994). Three different brucellosis strains have been isolated from a number of different marine mammals in the United

Kingdom including the common seal, harbor porpoise, common dolphin, Atlantic white-sided dolphin, striped dolphin, hooded seal, gray seal and European otter (Foster et al. 1996). A *Brucella sp*. has also been reported isolated from a Pacific bottlenose dolphin from California (Ewalt et al. 1994). The brucellosis isolates obtained from the marine mammals have been reported as members of the genus *Brucella*, however they do not match any known *Brucella* sp. and probably represent new, undescribed strains (Ewalt et al. 1994). Brucellosis infection could potentially have a major impact on health and reproductive success of infected marine mammal species.

Harbor seal serum was tested at the Washington Department of Agriculture (WDA) Microbiology Laboratory in Olympia for the presence of antibodies to *Brucella abortus* antigens supplied through the National Veterinary Services Laboratory (NVSL) in Ames, IA. Procedures used for testing samples followed standard protocols for *Brucella abortus* testing developed by NVSL. Interpretation of results of screening of exposure to *Brucella abortus* followed standards developed by the U.S. Department of Agriculture. Serum was screened for brucellosis using the Brucella Buffered Plate Agglutination test antigen (BAPA), the Brucellosis Card test (BBA), Rivanol, and complement fixation (CF) test. Supplemental testing was done on a small number of serum samples using Particle Concentration Fluorescence Immunoassay (PCFIA). A seal was considered positive when all tests (BAPA, BBA and Rivanol [>I:50]) were positive; a seal with one or more but not all tests positive was considered suspect.

For culture and isolation of brucellosis from dead harbor seals examined from Puget Sound beaches, tissue samples from lymph nodes, organs, bodily fluids, and parasites were collected in individually labeled, sterile whirl packs and frozen at –20°C. Selected sample were sent overnight on dry ice to NVSL, Ames, IA for bacterial culture and isolation. Samples were cultured for brucellosis by method described by Ewalt (1989).

Representative tissue samples were collected during gross necropsies by WDFW and preserved in 10% formalin. Tissues were submitted to Northwest ZooPath (Dr. M. Garner) for histopathology. Tissues were trimmed, embedded in paraffin, sectioned, and stained. Following histopathology, tissues in paraffin blocks were submitted to NVSL, Ames, IA for brucellosis immunohistochemistries.

Results

Calicivirus

All harbor seal samples from Gertrude Island screened for exposure to calicivirus by Oregon State University were negative (Table 1).

Influenza

All harbor seal samples from Gertrude Island screened for exposure to influenza viruses (Seal/Mass/1/80 (H7N7) and Seal/Mass/27/83 (H4N5)) at St. Jude Childrens Hospital were negative (Table 1).

Morbillivirus

All harbor seal samples from Gertrude Island screened for exposure to morbillivirus (CDV and PDV) at the various laboratories were negative (Table 1).

Table 1. Results of viral disease screening of harbor sea1s at Gertrude Island by age class.

DISEASE	PUPS	WEANERS	YEARLINGS	SUBADULTS	ADULTS	TOTALS
CALICIVIRUS						
Serology	0/0	0/3	0/12	0/24	0/50	0/89
Culture	0/0	0/3	0/13	0/23	0/54	0/93
INFLUENZA						
Serology	0/0	0/3	0/12	0/24	0/50	0/89
Culture	0/0	0/3	0/13	0/23	0/54	0/93
MORBILLIVIRUS						
Serology	0/0	0/17	0/1	0/34	0/30	0/82

Leptospirosis

Screening of Gertrude Island harbor seals for exposure to leptospirosis occurred from 1986 through 1997, with a total of 81 of 361 harbor seals tested (Table 2) showing low titres (<1:400). All seals tested prior to 1994 (n=93) were negative for exposure to leptospirosis. Of seals tested since 1994 (n=268), 81 (31%) had low leptospirosis titres (<1:400), primarily for *L. grippotyphosa*, indicating possible exposure but no active leptospirosis infection (Table 2). Two additional seals caught in 1997 from other areas had positive titres (>1:400), indicating presence of leptospirosis. One animal was an adult female caught in the Columbia River with titres of 1:800 for *L. pomona*. The other animal was an adult male caught in the Puntledge River near Courtney, British Columbia, with titres of 1:3200 for *L. pomona* as well.

Table 2. Leptospirosis titres in harbor seals sampled at Gertrude Island, 1986–1997

		Negative*		Suspect**		Positive***	
Age Class	n=	Females	Males	Females	Males	Females	Males
PUP	62	18	12	14	18	0	0
WEA NER	64	27	26	5	6	0	0
YEARLING	37	16	15	5	1	0	0
SUBADULT	68	20	38	4	6	0	0
ADULT	130	29	79	7	5	0	0
T0TALS	361	110	170	35	46	0	0

* NEGATIVE = No titres
** SUSPECT = Titres < 1:400
*** POSITIVE = Titres > 1:400

Brucellosis

A total of 263 seals were tested for evidence of exposure to brucellosis at Gertrude Island between 1994 and 1997 (Table 3). A total of 53 (20%) had suspect or positive titres for brucellosis. Twenty-nine (11%) had a suspect titre and 24 (9%) had a positive titre. Additional harbor seals captured in other areas of Washington also showed evidence of brucellosis exposure. Seals screened from haulout sites at Desdomona Sands (in the Columbia River) and Minor Island (in the Strait of Juan de Fuca) resulted in 3/22 (14%) and 14/77 (18%) with suspect or positive titres for brucellosis, respectively.

Table 3. Brucellosis titres in harbor seals sampled at Gertrude Island, 1994–1997.

		Negative*		Suspect**		Positive***	
Age Class	n=	Females	Males	Females	Males	Females	Males
PUP	42	22	16	2	2	0	0
WEANER	77	28	37	5	4	1	0
YEARLING	22	2	9	2	0	4	7
SUBADULT	35	10	10	2	6	3	5
ADULT	87	34	44	0	6	0	3
TOTALS	263	101	109	11	18	8	16

* NEGATIVE = All brucellosis tests negative
** SUSPECT TITRES = One or more but not all brucellosis tests (BAPA, BBA and Rivanol >I:50) positive
*** POSITIVE TITRES = All brucellosis tests (BAPA, BBA and Rivanol>I:50) positive.

For harbor seals screened for titres to brucellosis at Gertrude Island, the age classes that had the highest rates of suspect or positive titres were yearlings (6–18 months old) with 19/29 (65%), followed by subadults (18–48 months old) with 20/50 (40%). The age class with lowest percent of suspect or positive titres were adult animals (> 48 months) with 14/129 (11%). Of all the adult females screened for brucellosis titres in Washington, only one animal was seropositive. This adult female was caught in the Columbia River and showed evidence of having recently aborted based on vaginal distention.

Serum samples were also obtained from an additional 99 harbor seals found dead on Puget Sound

beaches and screened for evidence of exposure to brucellosis. A total of 18 (18%) of these animals showed suspect or positive titres for brucellosis. NVSL was able to isolate a *Brucella* sp. organism from four of six (67%) freshly dead animals where serology indicated exposure to brucellosis. The type of brucellosis isolated from Puget Sound harbor seals was biochemically similar to a *Brucella* species identified from a seal in the United Kingdom. However, subsequent DNA testing showed these were genetically distinct strains. The *Brucella* species found in Puget Sound harbor seals was isolated from 27 of 34 tissues and body fluids sampled, with heavy culture growth from samples collected from the lymph nodes, lungs, urinary bladder and feces (Table 4).

Immunohistochemistry techniques found positive brucellosis staining within the uterus and gut of the *Parafilaroides* sp. lungworms from two of the animals where a brucellosis organism was cultured and isolated. In addition to being present inside of the *Parafilaroides*, staining also revealed the presence of brucellosis in inflammatory cells and an abscessed area of the surrounding parachynema (Garner et al. 1997). There was also positive staining in some of the lymph node tissues as well.

Table 4. Tissues cultured for brucellosis from harbor seals stranded in Puget Sound

Tissue Sampled	96-7	96-9	97-4	97-9
Lymph nodes				
Sublingual	x	+		+++
Submandibular			++	
Supra scapular		+	++	+++
Sub scapular	x	+	++	+++
Inguinal	x	ng	++	+++
Mesenteric	x	+	++	+++
Iliac	x	ng	+	++
Renal			+	++
Splenic	x		+	++
Gastric	x		++	+
Mediastinal	x	ng	++++	++
Reproductive			+	++
Hepatic				++
Pulmonary			++++	++
Cardiac				++
Organs				
Tonsil			+	+
Thymus				+
Reproductive tract	ng	ng	++	ng
Liver	ng	+	+	ng
Kidney	ng	ng	++	ng
Spleen	ng	ng	+	++
Pancreas	ng	ng	++	ng
Lung	ng	+++	Pure growth	++
Synovial tissue			+	ng
Eye			+	ng
Feces			+++	ng
Urinary Bladder			++	

++++ Confluent growth (>1000 colonies)
+++- Heavy growth (100–999 colonies)
++ Moderate growth (10–99 colonies)
+ Sparse growth (<10 colonies)
ng No growth

Discussion

Monitoring the status and health of Puget Sound harbor seals has been one of the primary objectives of the WDFW's PSAMP research efforts. Annual health screening for a variety of diseases known to be either zoonotic or to have caused mass die-offs in marine mammals in other areas provides one mechanism to assess health risks to Puget Sound harbor seals. Evidence suggests Puget Sound harbor seals may have been exposed to leptospirosis, although the significance of low-level titres to this disease remains unknown. Regional harbor seal populations show evidence of exposure to a previously unknown strain of brucellosis that may be significant relative to other marine mammals and domestic livestock.

Serology screening indicates that Puget Sound harbor seals represent a generally naive population to diseases such as calicivirus, influenza virus and morbillivirus. The etiology and risks from these diseases relative to Puget Sound harbor seals remains unknown. Research has shown links between seals in areas of relatively high environmental contamination, failed immune response, and disease (Ross et al. 1996), which may also play a role in the health of Puget Sound harbor seals as well. In addition to health risks to Puget Sound harbor seals, the presence of brucellosis in marine mammals harvested by various Native American tribes in the Northwest poses an unknown human health risk related to potenial human exposure and infection. Marine mammal biologists, seal rehabilitators, and fishermen who have direct contact with pinnipeds may also be at risk of brucellosis infection.

Acknowledgments

Research activities were conducted under MMPA Scientific Research Permit No. 835. Funding and support for these research activities have been provided by the Washington Department of Fish and Wildlife; Puget Sound Ambient Monitoring Program; and National Marine Fisheries Service.

Thanks to all the agencies and individuals that have helped with harbor seal capture operations over the years, including personnel from the Washington Department of Fish and Wildlife; National Marine Mammal Laboratory; National Marine Fisheries Service; Oregon Department of Fish and Wildlife; Department of Fisheries and Oceans (Canada); and Cascadia Research.

A number of laboratories and researchers provided expertise in disease screening and interpretation of results, including: Washington Department of Agriculture, Olympia, WA; U.S. Department of Agriculture, National Veterinary Service Laboratory, Ames, IA; Northwest ZooPath, Snohomish,WA; Phoenix Central Laboratory, Everitt, WA; National Institute of Public Health and Environmental Protection (NIPHEP), Netherlands (Dr. A.Osterhaus); San Jose State University, Department of Biological Sciences (Dr. J. Boothby); Department of Pathology, Microbiology and Immunology, University of California, Davis (Drs. J. Stott, D.King and L. Landon); Department of Pathology, University of Guelph (Drs. P.Duignan and D. St. Aubin); U.S. Department of Agriculture, Plum Island (Dr. C. House); Oregon State University (Dr. A. Smith and Doug Skilling); and St. Jude Childrens Hospital, Memphis TN (Dr. V.Hinshaw).

A special thanks to all the many individuals who have supported WDFW's PSAMP-related harbor seal research efforts, particularly: Harriet Huber, John Pierce, Dave Nysewander and Scott Redman.

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